Development of a biological control program for Eurasian watermilfoil (*Myriophyllum spicatum*)

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15. SUBJECT TERMS
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Abstract
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List of keywords
Biological control, Eurasian watermilfoil, *Myriophyllum spicatum*, insects, pathogens, surveys, hybridization, aquatic weed

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Introduction and Background

Eurasian watermilfoil, *Myriophyllum spicatum* L., is the most important waterweed in the continental United States causing high costs in control efforts (Johnson and Blossey, 2002). Plants are rooted at the lake bottom and grow rapidly creating dense canopies (Aiken et al., 1979). High densities of Eurasian watermilfoil negatively affect wildlife and fish populations and make recreational use difficult or impossible (Johnson and Blossey, 2002, and references therein). In addition, species diversity and native macrophyte declines have been reported due to Eurasian watermilfoil invasions (Smith and Barko, 1990).

Myriophyllum spicatum reproduces by seed but vegetative reproduction by fragmentation is the most likely mode of spread (Smith and Barko, 1990, and references therein). Recent molecular analyses have shown that introduced *M. spicatum* is interbreeding with the holarctic *M. sibiricum* Komarov resulting in a hybrid with high regenerative capability (Moody and Les, 2002; in Press). Traditional control measures such as mechanical removal of the plants are labour-intensive and expensive, whereas chemical control has considerable side effects on other aquatic organisms and water quality. Three insects in North America have the potential to control *M. spicatum* two of which are accidental introductions from Europe (Johnson and Blossey, 2002). The native weevil *Euhrychiopsis lecontei* Dietz is commercially available. However, the success of the three insects is not consistent.

Myriophyllum spicatum is native to Europe, Asia, and North Africa, and appears to have been accidentally introduced into North America between the late 1800s and 1940 (Couch and Nelson, 1985; Nichols and Mori, 1971; Philippi, 1992). In 1965, a classical biological control program was initiated for *M. spicatum,* and overseas and US researchers surveyed parts of the native range for specialist natural enemies (insects and pathogens) for control of Eurasian watermilfoil in the US (Buckingham, 1998; Ghani et al., 1970; Spencer and Lekic, 1974). Surveys were made in former Yugoslavia, Pakistan, Bangladesh, and China, and more than 20 species were identified as feeding on *M. spicatum*. However, few were seriously investigated to determine their potential as biological control agents.

The following report reviews information on the taxonomy and indigenous distribution of *M. spicatum,* on insects associated with *Myriophyllum* spp. in Eurasia with a brief description of species with potential as biological control agents, and reviews collection methods. We subsequently identify research gaps and present a 2-year work program, at the end of which it should be possible to make an informed decision on the feasibility for biological control of *M. spicatum* in North America.

Objective 1: Critical assessment of the indigenous distribution of *Myriophyllum spicatum*

Taxonomy

The genus *Myriophyllum* belongs to the watermilfoil family, Haloragaceae, in the order Saxifragales. Only one other genus within the Haloragaceae occurs in eastern North America represented by the two species of mermaid weeds: *Proserpinaca palustris* L. and *P. pectinata* Lam. (Gleason and Cronquist, 1991). There has been much confusion regarding the taxonomic status and identity of *Myriophyllum* species. During a literature and internet survey we found nearly 70 accepted *Myriophyllum* species described worldwide. The centre of diversity of the genus *Myriophyllum* L. is in Australia, where about 40 species occur (Orchard, 1985). North America has eleven native species, plus introduced species from Europe (*M. spicatum*), Asia (*M. ussuriense* (Regel) Maxim.), and South America (*M. aquaticum* (Vellozo) Verdcourt) (USDA Plant Database). About 13 species are described from Asia, and only 2-4 species each from Africa, South America and Europe (Annex 1). However, since many of these species are difficult to distinguish from each other using morphological characteristics, a considerable number of misidentifications are likely to have been made in the past and some distribution maps might not be reliable.

A phylogenetic study including several of the North American *Myriophyllum* species indicates that *M. spicatum* is most closely related to the holarctic species *M. sibiricum* and *M. alterniflorum* L. There is also a relatively close relationship to *M. verticillatum* and the native South and North American disjunct *M. quitense* Kunth. All other native North American species analysed (*M. heterophyllum* Michaux, *M. laxum* Shuttleworth ex Chapman, *M. hippuroides* Nutt. ex Torr. & Gray, *M. tenellum* Bigelow, *M. pinnatum* (Walter) Britt., Sterns & Pogg, *M. farwellii* Morong, and *M. humile* (Raf.) Morong) are well separated from *M. spicatum* (Moody, 2004).

M. spicatum was recently recognized to hybridize with the holarctic *M. sibiricum* in North America (Moody and Les, 2002), and Moody and Les (in Press) has started to investigate the distribution of hybrid and parental populations in the USA. While *M. spicatum* and *M. sibiricum* can be distinguished by morphological characteristics related to leaf segments and the presence (*M. sibiricum*) or absence (*M. spicatum*) of turions (overwintering buds), hybrids overlap with both parents in leaf characters and lack turions, thus can only reliably be distinguished using molecular analyses (Moody and Les, in Press). It has been shown for other species that hybridization increases their invasive potential (Ellstrand and Schierenbeck, 2000), but whether this is true for the *Myriophyllum* hybrid has yet to be investigated. It is also unknown, whether the two species hybridize in Eurasia.

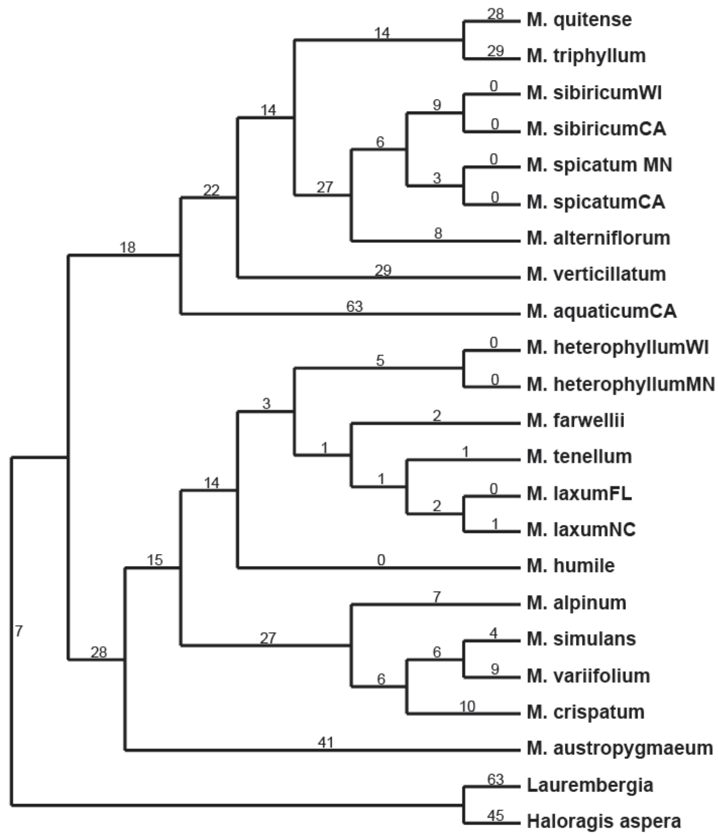

Fig. 1 Partial phylogenetic tree based on nrDNA ITS sequence data including most North American *Myriophyllum* ssp. (Moody, unpublished).

Distribution of *M. spicatum*

Myriophyllum spicatum is native in Eurasia and North Africa, occurring from Spain and UK in the west through to China and Japan in the east, and from Finland in the north to Morocco in the south (Meusel and Jäger, 1978). *Myriophyllum spicatum* is introduced and invasive in North America, South Africa, India, and Australia (Holm et al., 1979). In North America it is now present in 45 states and three Canadian provinces (USDA Plant Database).

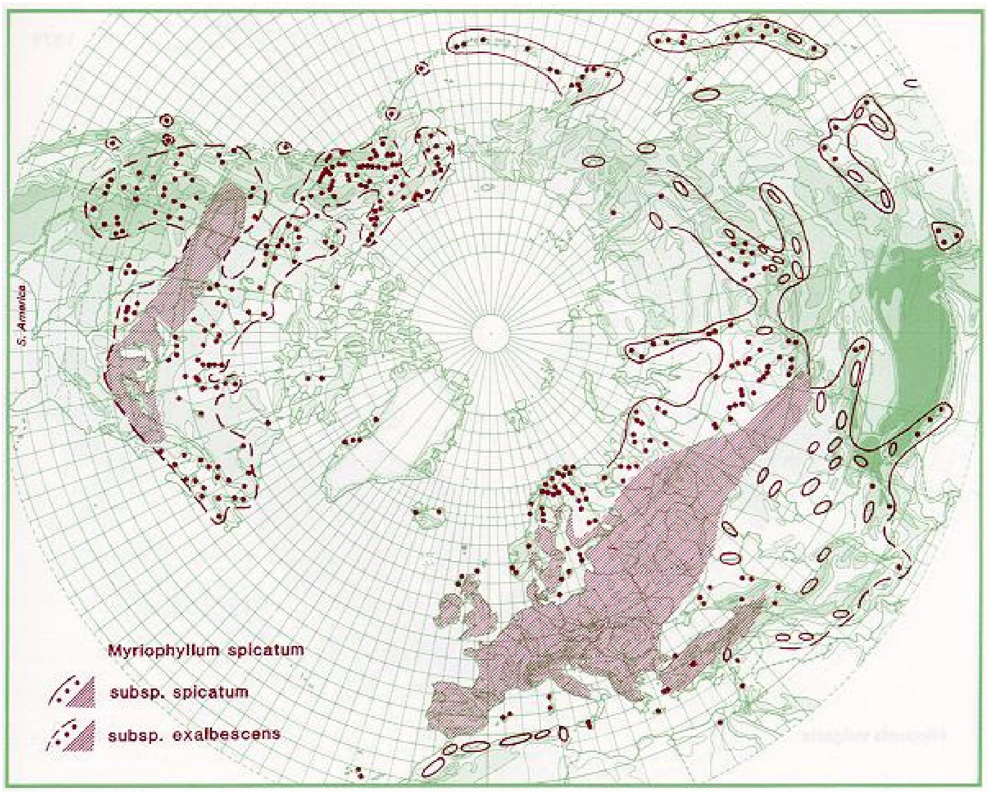

Fig. 2 Distribution map of *M. spicatum* and *M. sibiricum* (=*M. exalbescens*) according to Hultén and Fries (Hultén and Fries, 1986).

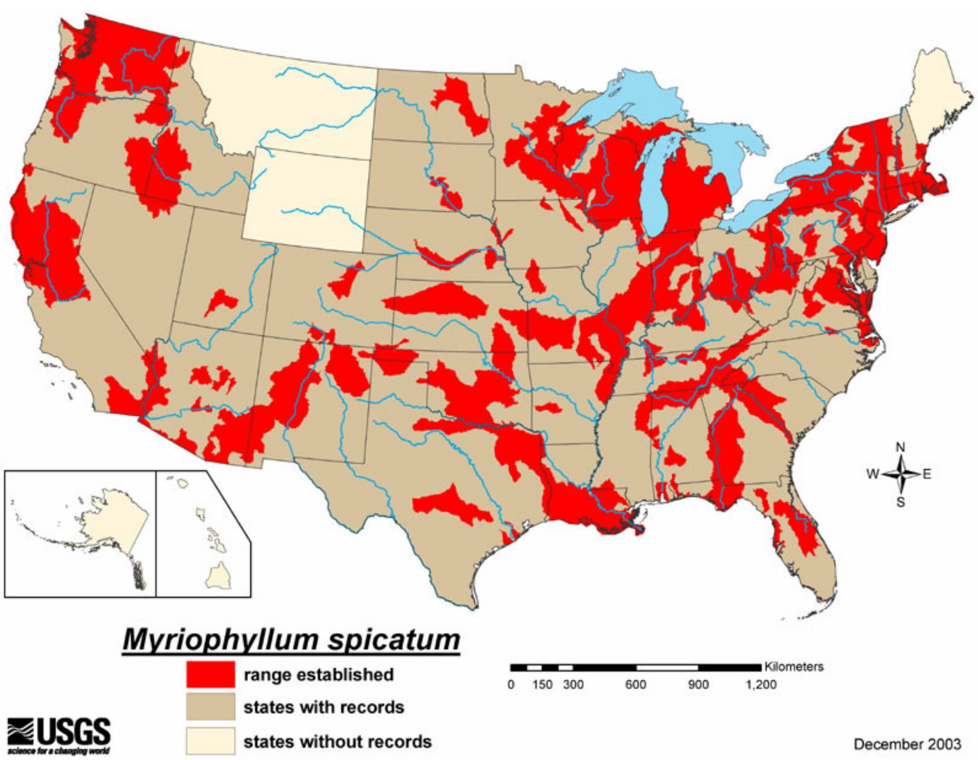

Fig. 3 Distribution of *M. spicatum* in North America (U.S. Geological Survey).

The closely related *Myriophyllum sibiricum* (= *M. exalbescens* Fernald) was formerly regarded as a purely native North American species. However, the species was first described in Russia by Komarov in 1914, and Linné probably included specimens of *M. sibiricum*, when he first described *M. spicatum*. Nevertheless, most of the European Floras do not mention *M. sibiricum* at all. Only a few recent publications recognize *M. sibiricum* as a holarctic species (Aiken and McNeill, 1980; Ceska and Ceska, 1986). Many reports about *M. spicatum* in Europe, especially in Northern Europe, might in fact be *M. sibiricum* (Faegri, 1982).

There is no clear area with significantly higher numbers of species of *Myriophyllum*, which overlaps with the indigenous range of *M. spicatum.* Such an area might have indicated a centre of diversification and evolution, and therefore an area to prioritise for surveys for biological control agents.

In summary it is clear that parallel to any biological control effort the occurrence and distribution of *M. spicatum, M. sibiricum* and their hybrid will need to be thoroughly investigated in both Europe and North America using molecular tools to prioritize survey areas and to be sure from which taxa insects are sampled.

Objective 2: Literature survey of insects associated with *Myriophyllum* spp. in Europe

A thorough survey of the European literature was carried out at CABI Europe - Switzerland, complemented by library visits and contacts with taxonomists, and of the Russian literature by our collaborator Dr. Margarita Dolgovskaya (Russian Academy of Sciences, Zoological Institute, St Petersburg, Russia). The European literature survey revealed 14 additions to the existing lists compiled by Ghani et al. (1970) for Pakistan and Bangladesh, Buckingham (1998) for China, Japan, Korea, and Spencer and Lekić (1974) for Yugoslavia. In total, 44 phytophagous insects have been associated with *Myriophyllum* spp. in Eurasia (Annex 2). The majority are Lepidoptera (n=12) and weevils (beetles in the family Curculionidae n=15). The latter is especially rich in aquatic weevils in the genus *Bagous* (n=6). Most insects are recorded as polyphagous, i.e. feeding on plants in different families. However, ten species are recorded to only feed on *Myriopyllum* spp. or the indication of other host plants needs to be verified by tests, which is the case for *Aristotelia* sp.? *subdecurtella* (Stainton) (Gelechiidae). There is no obvious pattern in which region the most specialized phytophagous insect species can be found.

Although only limited information is available for most of these species, none appears to be strictly monophagous, i.e. only feeding and developing on *M. spicatum.* However, due to their concealed development and their inaccessibility,

the phytophagous insect fauna of watermilfoils might have been underestimated. In addition, only a relatively small area of the total native range of Eurasian watermilfoil has been surveyed during relatively short windows in the growing season. It is therefore unlikely that the full spectrum of natural enemies associated with this plant has been captured

Eight of the insects recorded in the literature occur also in North America on *M. spicatum.* Some of these species appear to be native to North America and to have switched from their original hosts, e.g. *Euhrychiopsis lecontei*, which feeds on the native *M. sibiricum* (Sheldon and Creed, 1995); others may have been accidentally introduced from Europe (Buckingham et al., 1981). Three insects are particularly common in the U.S.: the naturalized pyralid moth *Acentria ephemerella* Denis & Schiffermüller, the native North American weevil *Euhrychiopsis lecontei*, and the Chironomid *Cricotopus myriophylli* Oliver which is most likely an accidental introduction since it occurs only in the distribution range of *M. spicatum* in the U.S. (MacRae et al., 1990). All three insect species can cause a decline of Eurasian watermilfoil in some lakes but not in others. One reason could be the partial resistance of hybrids to insect attack (Moody and Les, 2002).

Insect species with potential as biological control agents for Eurasian watermilfoil based on existing surveys

The most host-specific herbivores associated with *M. spicatum* in Europe and Asia are weevils in the genera *Bagous, Eubrychius*, and *Phytobius*, as well as a gelechid moth. A clear advantage of weevils as potential biological control agents is the fact that the feeding habitat of the adults is identical with that of the larvae resulting in a higher feeding pressure on the host plant. In contrast, only the immature stages of Lepidoptera damage the plants.

Bagous longitarsis Thomson

Bagous longitarsis Thomson has a Palaearctic distribution and occurs as far south as southern France and northern Italy (Dieckmann, 1983; Sprick, 2000). The weevil lives submerged on milfoils and feeds on the leaves from May onwards until August/September but overwinters outside the water (Dieckmann, 1983). Larval development is unknown (Sprick, 2000). *Bagous longitarsis* is exclusively known from watermilfoils in the field. In Germany, it has been collected from *M. verticillatum* (Dieckmann, 1983; Wimmer and Sprick, 2000), a holarctic species of watermilfoil which is widely distributed across Europe, and from *M. spicatum* (pers. communication Suikat and Gruschwitz, 1999, in Sprick 2000). During field surveys on watermilfoils in northern Germany, Wimmer and Sprick (2000) did not record *B. longitarsis* on *M. heterophyllum*. Contact has been made with these scientists and weevils could easily be collected and used in host specificity and preference tests.

***Bagous collignensis* (Herbst)**
This weevil occurs throughout Europe and Western Asia (Sprick, 2000). In the field, *B. collignensis* was so far sampled on *M. heterophyllum* (Wimmer and Sprick, 2000) and on *M. spicatum* (Sprick, 2000). According to Sprick (2000), *B. collignensis* is not associated with *Equisetum* as indicated in Dieckmann (1983). Again, individuals could easily be collected, since we have made contact with the scientists involved.

***Bagous geniculatus* Hochhuth**
Bagous geniculatus is a very widespread species and has been reported from Pakistan, Bangladesh (Ghani et al., 1970), Southern Europe, Central Asia and the Caucasus (Freude et al., 1983). *Bagous geniculatus* appears to be univoltine. The larvae feed inside the stems and roots (Ghani et al., 1970). The weevil requires submerged plants for feeding and sprouted plants outside the water for breeding (Ghani et al., 1970).

***Bagous vicinus* Hustache**
This weevil was sampled in Pakistan and Bangladesh. The larvae of *B. vicinus* bore into the stem and the roots and pupate in the soil (Ghani et al., 1970). Based on the observed developmental times, Ghani et al. (1970) assumed that *B. vicinus* has at least 10 to 12 generations per year.

Both *Bagous geniculatus* and *B. vicinus* caused considerable damage to submerged watermilfoil plants in Pakistan and Bangladesh (Ghani et al., 1970). Preliminary screening tests indicate that both species are specific to *Myriophyllum* or more narrowly within the genus (Ghani et al., 1970). However, native North American *Myriophyllum* spp. were not included in the host-range tests reported.

***Eubrychius velutus* Beck**
Eubrychius velutus Beck is an aquatic weevil distributed throughout Europe and northern Asia (Dieckmann, 1972). The weevil develops submerged in the meristem and outer portions of the plant and pupates in a cocoon near the shoot tip. Wimmer and Sprick (2000) observed that shoots of *M. heterophyllum* break easily near the cocoon. *Eubrychius velutus* is closely related to the native North American weevil *Eubrychius lecontei* which mines and pupates inside the stem of *M. sibiricum* and *M. spicatum* (Newman et al., 2006). During field investigations in Northern Germany carried out by Wimmer and Sprick (2000), *E. velutus* was the most abundant weevil recorded on the natives *M. spicatum* and *M. verticillatum* as well as on the naturalized *M. heterophyllum*. Larvae, pupae and adults of *E. velutus* were found on all three watermilfoil species (Wimmer and Sprick, 2000). Developmental rates and survival of *E. velutus* were similar on *M. heterophyllum* compared to the native hosts *M. spicatum* and *M. verticillatum* (Newman et al., 2006). Therefore, all three watermilfoils can be regarded as field hosts of *E. velutus*. However, multiple-choice host range tests would give an

indication as to whether *E. velutus* has feeding and oviposition preferences within the genus *Myriophyllum*.

***Phytobius* spp.**

Several *Phytobius* spp. are listed in Buckingham (1998) and Ghani et al. (1970), all of which feed at least temporarily on aerial shoots of watermilfoil. *Phytobius* (*Pelenomus*) *canaliculatus* Fahraeus is the only species recorded from Europe (Dieckmann, 1972) whereas three other species have been found in China (Buckingham, 1998) and Bangladesh (Ghani et al., 1970). All *Phytobius* spp. were recorded from *M. verticillatum* or other *Myriophyllum* spp. in the field and are thus not monophagous on *M. spicatum*.

***Aristotelia* sp. ? *subdecurtella* (Stainton)**

Larvae of this gelechid moth feed on the floral parts of *M. indicum* and *M. tuberculatum* (Ghani et al., 1970). Medvedev (1981) reports larval feeding by *A. subdecurtella* on *Lythrum salicaria* L. (purple loosestrife). Although some species of *Aristotelia* are known pests of crop plants, no other host records were available for *A. subdecurtella* (Ghani et al., 1970). Single-choice oviposition tests showed that no oviposition occurred on other plant genera when simultaneously exposed to *M. spicatum*. In no-choice larval transfer tests, complete larval development occurred on *Trapa bispinosa* Roxb. As mentioned for *B. vicinus* and *B. geniculatus*, this moth had not been exposed to native North American *Myriophyllum* spp. (Ghani et al., 1970).

Objective 3: Preliminary trials on collection and handling methods

To start assessing its European habitat, validate collecting methods and evaluate the logistics of working with *M spicatum* in the laboratory and greenhouse, we collected *M. spicatum* at two ponds located in the Swiss and French Jura in September 2006. These samples were carried out without special equipment. Plants were pulled out with a 1.5 m long hook from the shore. However, for systematic sampling, we will need rubber waders and an inflatable boat and longer tools to be able to pull out the plants from the ground level. Diving equipment should be tested particularly when sampling earlier in the year when plants are small or for sampling insect-damaged plants that remain small. Buckingham (1998) indicates further helpful tools like a bifocal head magnifier to examine damaged plants in the field. Some of the samples were transported back to the laboratory in plastic cylinders filled with water, some were wrapped in wet paper towels in plastic bags. During longer collection trips the storage of samples during transport will be difficult. Therefore, a mobile stereo-microscope will be essential to be able to analyze the samples directly at the field sites. We will also need to test further rearing techniques for the transport, as the methods used by Buckingham (1998) involved quite high mortality rates.

The only stem mining larvae found at this time of the year were chironomids. They were found on nearly every plant in different development stages. We were able to rear some individuals to adults.

In some stems we found empty pupal chambers, probably from a curculionid species. We also found two adults of an unidentified beetle. However, it is not clear whether it feeds or develops on *Myriophyllum*.

We were able to keep insects alive for several weeks in plastic vials and cups with the water changed weekly. However, for further rearing occasionally aerated aquaria and larger jars might be helpful. Buckingham et al. (1981) obtained highest survival rates during rearing in temperature cabinets at 18-22 °C and 16L:8D photoperiod.

We are now gaining experience in plant cultivation with several plants of *M. spicatum* grown from cut stem sections in plastic cylinders filled with water and a small layer of sand, mud or gravel. For future insect rearing we will probably use swimming pools and plastic or glass aquaria, in which plants potted in plastic cups can be placed.

Conclusions and research gaps

Only a limited area of the total native range has been surveyed

Based on results of previous field surveys and our additional literature search, no strictly monophagous species, i.e. only feeding and developing on *M. spicatum* have been found so far. However, only a relatively small area of the total native range of Eurasian watermilfoil has been surveyed during relatively short windows in the growing season. It is therefore unlikely that the full spectrum of natural enemies associated with this plant has been captured.

In Asia, surveys for arthropods carried out so far were limited to Pakistan, Bangladesh, the northern parts of China and two short visits of one week each to Japan and Korea. Buckingham (1998) therefore concluded that substantially more work would be needed before concluding that no suitable insect natural enemies occur in these countries. Central Asian countries, such as Kazakhstan, Uzbekistan and Mongolia have not been surveyed at all yet.

The European survey for phytophagous insects was only done in former Yugoslavia. Additional surveys should therefore be conducted in France, Spain, Switzerland, Germany, Sweden, Poland, Romania, Russia, and the Ukraine. Furthermore, the roots of *M. spicatum* were probably never systematically sampled and analysed for insect damage during previous samplings (Buckingham, 1998).

Surveys for pathogens were restricted to Western Europe and China. North Africa has not been surveyed at all. Although this report was not intended to

specifically include pathogens as potential biological control agents for *M. spicatum*, future work should try, where possible, to combine insect and pathogen surveys. This would not only be most cost-effective, but it has also been our experience that such an approach is synergistic in understanding the interactions of fungi, insects and the damage that they cause, and reducing the chance of obscure types of damage being overlooked. In addition, pathogens can be even more specific than insects, which could be important in the case of *Myriophyllum* (see below).

Limited host-specificity tests conducted with potential agents

Only limited or no host-specificity tests were conducted with potential agents selected or found during previous surveys. At the time of these studies, there was no great concern over the possibility of indigenous plant species being attacked by introduced weed biological control agents. This is no longer the case. Thus, tests did not include North American *Myriophyllum* species, which would obviously be the most critical species to test now. A few critical North American *Myriophyllum* species as well as the two representatives of the only other genus within the same family (Haloragaceae), the mermaid weeds: *Proserpinaca palustris* and *P. pectinata* should therefore be obtained, and immediately included in test of any potential biological control agent selected.

Since laboratory tests could underestimate host specificity of some agents, field surveys on other *Myriophyllum* species would give additional valuable information regarding the field host range of the existing herbivores. For instance, the naturalized North American twoleaf milfoil, *M. heterophyllum,* and three watermilfoil species which are native to Europe and North America, i.e. *M. alterniflorum, M. verticillatum,* and *M. sibiricum*, should also be sampled during surveys in Europe.

In addition, any potential biological control agent will need to be tested against the hybrid and its two parental species, *M. spicatum* and *M. sibiricum,* preferably using material from both continents to determine potential preferences for any of these types or their potential resistance to insect attack.

At this stage, there is no clear indication of the level of specificity or preference, within the genus *Myriophyllum* that might be expected from the potential biocontrol agents selected. However, preferences have been reported for the insects present in North America that feed on *Myriophyllum* spp. Furthermore, *Lysanthia* n. sp. (Chrysomelidae), a leaf-feeding beetle introduced from South America to control parrot's feather, *M. aquaticum* in South Africa only develops and feeds on *M. aquaticum* but not on *M. spicatum*, the only *Myriophyllum* species tested (Cilliers, 1999). Thus, a degree of specificity or preference within the genus is certainly possible.

Occurrence of hybrids between M. spicatum *and* M. sibiricum

The fact that the invasive *M. spicatum* and the holarctic *M. sibiricum* hybridize in North America, potentially resulting in an even more invasive form, needs to be carefully considered when developing a biological control program.

The following questions are of particular interest:

- how widespread and abundant is the hybrid between *M. spicatum* and *M. sibiricum* in North America?
- is the hybrid really more invasive than *M. spicatum*?
- do the parental species and their hybrid differ in habitat preferences / environmental conditions?
- do the two species also hybridize in Eurasia?

To try and answer these questions, we contacted Dr. Michael Moody (Indiana University), who has been working on the genetics of *Myriophyllum* spp. and their phylogenetic relationships since 2000. It was he with co-workers who demonstrated the existence of *Myriophyllum* hybrids. In addition to the above listed questions, Dr. Moody is also interested in examining the genetic variability of *M. spicatum* and *M. sibiricum* within and between North American and Eurasian populations, and to identify the origins of North American populations of *M. spicatum* and the hybrid watermilfoil. Co-operation with Dr. Moody will be of paramount importance, not only for the development of a biological control program, but also for the management of invasive *Myriophyllum* spp. in North America in general.

The collection of Eurasian material for molecular analysis can easily be combined with our planned insect and pathogen surveys, while the collection of North American material will be organized by Dr. Moody. Expenses to conduct the analyses are only included for the European samples (see Annex 3). Dr. Moody will have his own resources to analyse additional NA material and tackle the other questions listed above.

Objective 4: Suggested work program for 2007 and beyond

Considering the unpredictability of the outcome of more extended surveys, we suggest to split the project into two phases. Phase 1 will consist of a two year period, during which the above mentioned research gaps will be filled. At the end of phase 1 we will be able to determine whether biological control of Eurasian watermilfoil is a feasible and valid option and produce a priority list of potential agents. Results will provide the basis to develop a realistic, well founded five-year project plan (phase 2). At the beginning of Phase 2 we would develop a comprehensive test plant list in co-operation with North American counterparts to be submitted to the Technical Advisory Group (TAG) for comment. Prioritized

biological control agents would be tested against species on this list and - depending on results - a petition for field release of at least one biological control agent could be prepared.
A tentative budget is included in Annex 3.

Proposed work program for Phase 1

Year 1:

- Obtain a selection of native North American and holarctic *Myriophyllum* spp., including *M. alterniflorum, M. farwellii, M. heterophyllum, M. humile, M. pinnatum, M. sibiricum*, *M. tenellum*, and *M. verticillatum* as well as two other hydrophytes in the same family, i.e. *Proserpinaca palustris* and *P. pectinata* for preliminary host range testing of these key species;
- Obtain different populations of the hybrid (*M. spicatum* x *M. sibiricum*) and parental species from North America and of parental species from Eurasia to test potential preferences of selected biological control agents;
- Develop a standardized sampling protocol including the root system of plants;
- Refine existing techniques for rearing and handling of plants and insects, and develop new methods as needed;
- Collect the three weevil species for which sites relatively close the Centre are known (*Bagous collignensis, B. longitarsis, Eubrychius velutus*) and conduct host-range tests with some critical test plant species;
- Conduct field surveys in areas previously not sampled before, e.g. Spain, France, Sweden, Poland, Russia, central Asia; collect insects and pathogens on *M. spicatum* and other *Myriophyllum* species if present;
- Start rearing and investigations on the biology of the most promising herbivores and pathogens;
- Start assessing occurrence and distribution of *M. spicatum*, *M. sibiricum*, and potential hybrids in Europe in collaboration with Dr. Moody (DNA samples, herbarium specimens);

Year 2:

- Continue obtaining native plant material;
- Continue and extend field surveys, especially in less accessible areas, e.g. Pakistan, Mongolia;
- Conduct host-specificity tests against a set of critical test species (see above) with the most promising herbivores and pathogens;
- Conduct preference tests against the hybrid and parental species (see above) with the most promising herbivores and pathogens;

- Continue assessing occurrence and distribution of *M. spicatum*, *M. sibiricum*, and potential hybrids in Europe in collaboration with Dr. Moody (DNA samples, herbarium specimens);

It should be noted that, as with all classical biological weed control programs, there are factors that can adversely affect progress. Therefore, the work plan outlined above is based on the following reasonable assumptions:

- The annual required funding will be available in a timely manner, that is prior to start of the field season (April);
- The exchange rate used (1 $U.S. = 1.1 SFr) will not worsen;
- The political situation in the proposed survey areas will remain stable:

At the end of each year a detailed Annual Project Report will be provided to project sponsors and interested parties. The work plan for each year will be adjusted annually and adjustments will be included in the Annual Project Report. Project progress will be discussed at least once a year in person and if necessary additional telephone conference calls with project sponsors.

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Appendix

Annex 1 Known *Myriophyllum* species in Eurasia, North and South America, and Africa (Eu = Europe, As = Asia, NA = North America, SAm = South America, Afr = Africa, Aus = Australia)

Species	Synonym	Origin	Remarks	References
M. spicatum L.	*M. magdalenense*?, *M. montanum* Martrin-Donos?	Eu, As, NAfr	Introduced in NA, Aus, SAfr	Dong et al. 2002, Aiken 1981
M. alterniflorum L.	*M. montanum* Martrin-Donos	Eu, NA	Sterile confused with *M. sibiricum*, distribution in overlapping areas unclear	Ceska & Ceska 1986, Dong et al. 2002, Aiken 1981
M. verticillatum L.	*M. limosum* Hect., *M. siculum* Gusonne, *M. spicatum* Gmelen, *M. pectinatum DC*	Holarctic		Meusel & Jäger 1978, Dong et al. 2002, Aiken 1981
M. sibiricum Komarov	*M. exalbescens* Fernald, *M. spicatum* spp. *squamosum* Laest. ex Hartman fil, *M. magdalense*	Holarctic?		Ceska & Ceska 1986, Aiken 1981, Löve 1961, Faegri 1982
M. isoetophyllum Kom.		Russia		Komarov 1929
M. bonii Tardieu		Asia		Orchard 1985
M. dicoccum F.Muell.		Asia (China), Aus		Les et al. 2003, Dong et al. 2002
M. exasperatum D.Wang, D.Yu&Z.Y.Li		Asia (China)	New description	Dong et al. 2002
M. indicum Willd.	*M. intermedium*	Asia (Sri Lanka, India)		Dong et al. 2002
M. oliganthum (Wight & Arn) F.Muell.	*M. intermedium*, *Haloragis oligantha*?	Asia (Sri Lanka, India)	Endemic?	Nijalingappa 1973
M. oguraense Miki		Asia (Japan, China)		Yu et al. 2002
M. tetrandrum Roxb.	*M. indicum* ?	Asia		Dong et al. 2002
M. siamense (Craib) Tardieu		Asia		Orchard 1985

Species	Synonym	Origin	Remarks	References
M. tuberculatum (Red)	*M. tetrandrum*, *M. indicum*, *M. spathulatum* Blatter & Hallb.	Asia		Dong et al. 2002
M. ussuriense (Regel) Maxim.	*M. japonicum*	Asia	Introduced in NA	Ceska & Ceska 1986, Les et al. 2003, Dong et al. 2002
M. farwellii Morong		NA		Aiken 1981
M. heterophyllum Michaux		NA	Introduced in Europe	Aiken 1981
M. hippuroides Nutt. ex Torr. & Gray	*M. mexicanum* S. Watson?, *M. scabratum* C. and S.?	NA		Aiken 1981
M. humile (Raf.) Morong	*M. ambiguum* Nutt. ?, *M. capillaceum* Torr., *M. procumbens* Bigelow	NA		Aiken 1981
M. laxum Shuttleworth ex Chapman		NA		Aiken 1981
M. tenellum Bigelow	*M. nudum* La Pilaye ex DC.?	NA		Aiken 1981
M. pinnatum (Walter) Britt., Sterns & Pogg.	*M. eggelingi* Hort. ex A.Chev. ??? *M. scabratum* Michaux	NA, Central America		Aiken 1981, Proctor 1982
M. quitense Kunth	*M. elatinoides* Gaud., *M. chiquitense* Meyen., *M. ternatum* Gauichaud., *M. titikaense* Remy, *M. viridescens* Gill.	NA, SAm		Orchard 1981
M. aquaticum (Vellozo) Verdcourt	*M. brasiliense* Cambess., *M. proserpinacoides* Gill.	SAm	Introduced in NA, Europe, China, SAfr, Aus	Orchard 1981, Dong et al 2002, Aiken 1981
M. mattogrossense Hoehne	*M. brasiliense*?	SAm		Orchard 1981
M. sparsiflorum Wright		Cuba	Extinct in Cuba 1999?	Urquiola Cruz & Betancourt Gandul 2000
M. mezianum Schindler		Afr (Madagaskar)		Orchard 1981

Annex 2 Results of a literature survey for phytophagous insects associated with *Myriophyllum* spp. in Europe and Asia (grey highlighting indicates potentially genus-specific species; *, species present in NA).

Name	Host range	Feeding niche, biology	Distribution	References
LEPIDOPTERA				
Crambidae				
**Acentria nivea* Olivier (= *A. ephemerella* Denis & Schiffermüller)	Polyphagous	Stem and leaf tissue. First instar larvae mine the food plant, then caterpillars feed on plants, cutting stems and removing leaves, live under water, larvae overwinter	Europe, Europ. part of former Soviet-Union (except N), Siberia. Accidentally introduced into NA	Rimskii-Korsakov 1940, Pavlovskii & Lepneva 1948, Gaevskaya 1966, Medvedev 1986, Creed & Sheldon 1994, Lvovskii 2001
Cataclysta lemnata L.	Polyphagous on various vascular water and hydrophyte plants	Young larvae in mines, then feed on leaves and cut stems	Europe, Europ. part of former Soviet-Union (except N), Caucasus, Central Asia	Kashkin 1959, Lekić & Mihajlovic 1970, Spencer & Lekić 1974, Medvedev 1986, Lvovskii 2001
**Nomophila noctuella* (Denis & Schiffermüller)	Polyphagous	Above-water defoliator	Pakistan, Europe, Europ. part of former Soviet-Union, Central Asia, N. America	Ghani et al. 1970, Spencer & Lekić 1974, Medvedev 1986, Lvovskii (pers. comm.)
Nymphula sp.	Probably polyphagous	Root and stem borer	Pakistan	Ghani et al. 1970
Nymphula (=Parapoynx) stagnata Donovan	Polyphagous (e.g. *Sparganium, Potamogeton, Nupha*)	Young larvae in mines, then larvae boring in leaves and stem, overwinter	Europe, Europ. part of former Soviet-Union (exept N), southern Siberia, Caucasus, Kazakhstan, Central Asia, Far East, Japan	Lekić & Mihajlovic 1970, Spencer & Lekić 1974, Lvovskii 2001
Elophila (=Nymphula, Hydrocampa) nymphaeata L.	Polyphagous on various vascular aquatic plants	Larvae live inside the plant, young larvae in mines, then feed on leaves, stem or flowers, larvae overwinter	Europe, Europ. part of former Soviet-Union (exept N), Siberia, Caucasus, Kazakhstan, Far East, Mongolia, Japan, China	Kashkin 1959, Lekić & Mihajlovic 1970, Spencer & Lekić 1974, Lvovskii 2001
Parapoynx stratiotata L. (=*Paraponyx stratiotatum*)	Polyphagous (*Trapa natans, Stratiotes aloides, Pontamogeron, Geratophyllum*, *Gallitriche*)	Larvae live in the cases on water suface, feed on fresh leaves, more rarely on decaying plants, overwinter	Europe, Europ. part of former Soviet-Union (exept N), Siberia, Caucasus, Kazakhstan, Far East	Pavlovskii & Lepneva 1948, Kashkin 1959, Gaevskaya 1966, Spencer & Lekić 1974, Medvedev 1986, Lvovskii 2001
Parapoynx (=Nymphula) vittalis Bremer (Park)	Polyphagous (*Hydrilla verticillata, M. verticillatum*)	Feeds on submerged leaves.	Russian Far East (Amur region, Khabarovskii kray, Primorskii kray), China	Buckingham 1998, Lvovskii 2001
Paraponyx nivalis Denis&Schiff.	Polyphagous, feeding on *M. spicatum* is rare	Leaves	Europe, Europ. part of former Soviet-Union	Lekić & Mihajlovic 1970, Spencer & Lekić 1974, Medvedev 1986

Name	Host range	Feeding niche, biology	Distribution	References
Gelechiidae				
Aristotelia sp. *? subdecurtella* (Stainton)	*M. indicum, M. tuberculatum,* no oviposition on other plant genera in single-choice oviposition tests, development on *Trapa bispinosa* in no-choice feeding tests. Larvae feed on *Lythrum salicaria*	Above-water feeder on the flowers	Lowland Bangladesh, W. Europe, West of Europ. part of former Soviet-Union	Ghani et al. 1970, Medvedev 1981
Noctuidae				
Agrotis segetum Schiff	Polyphagous		Pakistan	Ghani et al. 1970
Spodoptera exigua Hb.	Polyphagous		Pakistan	Ghani et al. 1970
COLEOPTERA				
Chrysomelidae				
Macroplea appendiculata Panz. (=*Haemonia appendiculata* Latr.)	Polyphagous (*M. spicatum, Potamogeton, Ranunculus,* various vascular water plants)	Adults feed on leaves, stem and larvae on roots	Europe, Europ. part of former Soviet-Union, Siberia, Kazakhstan, Central Asia, Algir	Freude et al. 1966, Tarbinskii & Plavilschikov 1948, Bei-Bienko 1965, Ogloblin & Medvedev 1971, Kuticova & Starobogatov 1977, Medvedev & Zaitzev 1978, Lopatin 1986, Medvedev 1988, Dubeshko & Medvedev 1989, Kireychuk & Ben'kovskii 2001
Macroplea mutica Fabricius	Polyphagous (*M. spicatum*, various vascular aquatic plants)	Feed on plant tissue	N-western Europe, Europ. part of former Soviet-Union, Siberia, Caucasus, Kazakhstan, Central Asia, Far East (South), Mongolia, Japan	Lopatin 1977, 1986, Bei-Bienko 1965, Medvedev 1982, Medvedev & Dubeshko 1992, Kireychuk & Ben'kovskii 2001
Macroplea pubipennis	*Myriophyllum, Potamogeton, Zanichellia*		Northern Europe	Nilsson 1996
Macroplea sp.	*Myriophyllum, Potamogeton*	Larvae in shelters attached to lower stem and roots but feeding damage not elucidated	China	Buckingham 1998
Donacia sparganii Ahrens	*Sparganium, Myriophillum, Butomus*	Larvae feed on roots, adults feed on leaves	Nothern and Central Europe, Central part of Europ. part of former Soviet-Union, Western Siberia, Far East (South), Mongolia, Japan	Kireychuk & Ben'kovskii 2001

Name	Host range	Feeding niche, biology	Distribution	References
Neohaemonia voronovae L.Medvedev	*M. spicatum, Potamogeton*	Larvae feed on roots, adults feed on leaves	Mongolia, ?Siberia	Medvedev 1977, Medvedev & Voronova 1979, Medvedev 1982, Korotyaev (pers.comm.)
Curculionidae				
Bagous collignensis (Hbst.)	*M. spicatum*, *M. heterophyllum*	Feed under water, larvae in stem	Europe, Anatolia	Koch 1992, Wimmer & Sprick 2000
Bagous geniculatus Hochhuth (= *Ephimeropus geniculatus*)	*M. spicatum, M. indicum, M. tuberculatum,* no oviposition on plants outside *Myriophyllum* in sequential no-choice tests	Root and stem borer, feeds on submerged plants, breeds on sprouting plants outside the water	Pakistan and lowland Bangladesh, Southern Europe, Caucasus, Central Asia, France, Hungary, Austria (Neusiedlersee)	Ghani et al. 1970, Korotyaev (pers. comm.), Freude et al. 1983
Bagous longitarsis Thomson	*Myriophyllum* spp., *Myriophyllum verticillatum*, feeds only in captivity on *Ceratophyllum,* not found on *M. heterophyllum*	Adults feed on leaves under water, adults live under water V-VIII	palaearctic (towards southern France and northern Italy)	Dieckmann 1983, Wimmer & Sprick 2000, Sprick 2000
Bagous myriophylli O'Brien	*M. spicatum, M. verticillatum*	Submerged, larvae tunnel in stems, adults make holes	China	Buckingham 1998
Bagous nodulosus Gyllenhal	Feeding on *M. spicatum* is supposedly occasional, feed mostly on *Butomus umbellatus,* but was also reported from *Caltha palustris.*	Stems	Europe, Siberia, Central Asia, Mongolia	Lekić & Mihajlovic 1970, Isaev 1994, Korotyaev (pers. comm.)
Bagous vicinus Hust.	*M. spicatum, M. indicum, M. tuberculatum*	Feed mainly on emergent flower-stalks	Pakistan, Bangladesh	Ghani et al. 1970
Eubrychius (=Lithodactylus,=Rhynchaenus) velutus (Beck)	*M. spicatum, M. verticillatum, M. heterophyllum*, *Potamogeton* sp.	Feed under water V-IX, larvae feed the young leaves near the shoot tip, pupation on the plant in a cocoon, overwinter on land under foilage	Europe, Middle Asia, North America questionable (*Eubrychiopsis lecontei* is morphologically slightly different), Mongolia, Russian Far East	Dieckmann 1972, Bei-Bienko 1965, Gaevskaya 1966, Isaev 1994, Egorov 1988, Korotyaev (pers. comm.), Wimmer & Sprick 2000
Eubrychius sp., it might be *E. velutus* (Buckingham 1998)	*Myriophyllum verticillatum, M. spicatum*?	Submerged, larvae and adults feed on leaves, esp. near and on meristem	China	Buckingham 1998
**Phytobius leucogaster* Marsham (= *Litodactylus leucogaster* (Marsham), *Phytobius griseomicans, L. myriophylli* Gyllenhall)	*M. spicatum, M. verticillatum, M. heterophyllum.* Probably identical with *Litodactylus griseomicans* (Schwarz) and *Phylobius albertanus* from NA	In water V-IX, larvae feed the young leaves near the shoot tip, overwinter on land under foliage, larvae and adults feed on leaves, stem and buds, probably feeding on emerse plant parts	Holarctic (Europe, Middle Siberia, Kazakhstan, Central Asia, North America)	Dieckmann 1972, Tarbinskii & Plavilschikov 1948, Pavlovskii & Lepneva 1948, Bei-Bienko 1965, Gaevskaya 1966, Isaev 1994, Egorov 1988, Korotyaev (pers. comm.), Wimmer & Sprick 2000

Name	Host range	Feeding niche, biology	Distribution	References
Pelenomus (=*Phytobius*) *canaliculatus* Fahraeus	*M. spicatum, M. verticillatum,* records on *Potamogenton natans* L. and *Polygonum mite* Schrk. have to be verified by tests	V-VIII only on aerial shoots, not under water, larvae feed on leaves	Europe (except Mediterranean), Japan, Europ. part of former Soviet-Union, Siberia, Kazakhstan, Mongolia	Dieckmann 1972, Tarbinskii & Plavilschikov 1948, Isaev 1994, Korotyaev (pers. comm.)
Phytobius sp. 1	*M. spicatum, M. verticillatum*	Adults and larvae feed on the flowers, they also eat meristems and leaflets on submerged shoots, cocoon in the stem	China	Buckingham 1998
Phytobius sp. 2	*M. spicatum* ?, *M. verticillatum*	Adults and larvae feed on the flowers, almost identical to *Phytobius* sp. 1 but slight morphological differences, cocoon attached to the leaves	China	Buckingham 1998
Phytobius sp.	*M. indicum, M. tuberculatum, M. spicatum,* adult feeding on *Polygonum hydropiper* in the field when *Myriophyllum* is submerged but no oviposition	Larvae and adults are flower feeders, multivoltine	Lowland Bangladesh	Ghani et al. 1970
Rhinoncus albicinctus Gyllenhal	Feeding on *M. spicatum is* occasional, mainly on *Polygonium amphibium* and related species	Larvae are stem-miners	Europe, Europ. part of former Soviet-Union, Siberia, China	Bei-Bienko 1965, Lekić & Mihajlovic 1970, Spencer & Lekić 1974, Korotyaev (pers. comm.)
**Tanysphyrus lemnae* Payk.	Polyphagous, was recorded mainly on *Lemna, Spirodela, Caltha*	Adults and larvae feed on leaves	Europe, Europ. part of former Soviet-Union, Siberia, Far East, Japan, N. America	Bei-Bienko 1965, Gaevskaya 1966, Spencer & Lekić 1974, Isaev 1994, Korotyaev (pers. comm.)
HOMOPTERA				
Aphididae				
**Aphis nasturtii* Kaltenbach	Polyphagous, *M. spicatum,* many plants including potato, tomato, pumpkin	Feed on stem	Europe, Europ. part of former Soviet-Union, Central Asia, N. America	Tarbinskii & Plavilschikov 1948, Lekić & Mihajlovic 1970, Spencer & Lekić 1974, Krizhanovskii 1974.
Rhopalosiphum nymphaeae L.	Polyphagous, *M. spicatum, Trapa natans,* other vascular water plants, at the first half of summer on *Rosaceae*	Sap-sucker, feed on stem	Pakistan, Bangladesh, almost whole world	Ghani et al. 1970, Mordvilko 1929, Lekić & Mihajlovic 1970, Spencer & Lekić 1974, Bei-Bienko 1964, Kryzhanovskii 1974
DIPTERA				
Chironomidae				
**Cricotopus myriophylli* Oliver	*Myriophyllum spicatum*	Stem tip destroying the apical	China, Korea, Inner	Buckingham 1998

Name	Host range	Feeding niche, biology	Distribution	References
(Oliver)		meristem	Mongolia, North America	
**Cricotopus sylvestris* (Fabricius)	Polyphagous, various vascular water and hydrophyte plants	Young larvae in leave mines, feed on fragments of living and dead plant tissue, periphytic algae and small animals	North America, almost whole world, including former Soviet-Union	Gosteva 1950, Shilova 1955, Konstantinov 1958, Gaevskaya 1966, Pankratova 1970, Spencer & Lekić 1974
Endochironomus ex.gr.dispar Meig.	Polyphagous, associated with various vascular water plants	Larvae feed on detritus, small animal reminds, also on fresh and dead plant tissue	Europe, Europ. part of former Soviet Union	Konstantinov 1958, Starostin 1992
Tanytarsus ex.gr. Lauterborny Kieff.	Polyphagous, associated with various vascular water plants	Larvae feed on fresh and dead tissue of plants, detritus, animals	Europe, Europ. part of former Soviet Union, Central Asia	Konstantinov 1958, Starostin 1992
TRICHOPTERA				
Leptoceridae				
**Mystacides (=Phryganea) longicornis L.*	Polyphagous, various water plants	Larvae feed on fresh and decaying plant tissue	Europe, Europ. Part of former Soviet Union, Siberia (to Kamchatka), Kazakhstan, N.America	Lepneva 1964, 1966, Gaevskaya 1966, Kuticova & Starobogatov 1977, Ivanov et al. 2001
?Leptocerus sp.		Larvae on leaves, not evaluated	China	Buckingham 1998
Hydropsychidae				
Indet.		Larvae on leaves, not evaluated	Japan	Buckingham 1998
Phryganeidae				
Phryganea grandis L.	Supposedly polyphagous on algae and on vascular plants. Seasonal change of food specialization. At the end of the summer they feed on larvae of other insects	Larvae feed on plant tissues	Europe, Europ. part of former Soviet Union, Eastem Siberia	Lepneva 1964, 1966, Kashkin 1958, Ivanov et al. 2001
Phryganea bipunctata Retzius (=Ph. striata auctorum)	Polyphagous on algae and on vascular plants	Larvae feed on plant and small invertebrates, *Myriophyllum* leaves are used to make a case for the first instar larva	Europe, Europ. part of former Soviet Union, Siberia, Far East, Mongolia	Lepneva 1964, 1966, Pavlovskii & Lepneva 1948, Ivanov et al. 2001

Annex 3: Proposed Budget for Phase 1

2007

Exchange rate used: 1 $ US = 1.1 SFR	
Staff costs	
Research Scientist	34,364
Lab Technicians	12,182
Garden Technician	6,395
External collaborators	3,000
Subtotal	**55,940**
Survey costs	
Europe	3,000
Russia	4,000
Asia	5,000
Subtotal	**12,000**
Other costs	
Material and Consumables CABI	5,500
Material and Consumables Moody	4,500
Identifications	2'000
Subtotal	**12,000**
Overheads @ 33%	26,380
Total	**106,320**

2008

Exchange rate used: 1 $ US = 1.1 SFR	
Staff costs	
Research Scientist	35,738
Lab Technicians	12,669
Garden Technician	6,650
External collaborators	3,000
Subtotal	**58,058**
Survey costs	
Europe	3,000
Russia	4,000
Asia	5,000
Subtotal	**12'000**
Other costs	
Material and Consumables CABI	5,500
Material and Consumables Moody	4,500
Identifications	2'000
Subtotal	**12,000**
Overheads @ 33%	27,079
Total	**109,137**